

中国东南沿海四种拟菱形藻生长特性与毒素检测研究

学校编码: 10384

学号: 200426017

分类号_____密级_____

UDC _____

厦 门 大 学

硕 士 学 位 论 文

中国东南沿海四种拟菱形藻生长特性与毒素检测研究

Growth characteristics and toxin determination of four species of *Pseudo-nitzschia* from Southeast China Sea

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论文提交日期: 2007 年 7 月

论文答辩时间: 2007 年 8 月

学位授予日期: 2007 年 9 月

答辩委员会主席: 郑天凌

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2007 年 8 月

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厦门大学博硕士论文摘要库

目 录

中文摘要.....	I
英文摘要.....	II
第一章 前言	1
1 拟菱形藻的分类学研究历史与现状	1
2 神经性贝毒软骨藻酸的来源、性质及分析方法	1
2.1 软骨藻酸的来源.....	1
2.2 软骨藻酸的性质及引发的症状.....	2
2.3 神经性贝毒软骨藻酸的分析方法.....	4
3 拟菱形藻与软骨藻酸研究进展	5
3.1 有毒拟菱形藻赤潮导致的 DA 污染.....	5
3.2 影响拟菱形藻产生 DA 的因素.....	7
4 国内拟菱形藻研究现状	10
5 本论文研究的内容、目的和意义	12
第二章 材料与方法	13
2.1 实验材料	13
2.2 实验方法	14
2.2.1 藻种分离.....	14
2.2.2 藻种培养.....	14
2.2.3 样品的处理与分析.....	15
2.3 数据处理与分析.....	18
2.4 主要仪器设备.....	19
第三章 结果与分析	20
3.1 藻种的分离与纯化	20
3.2 尖刺拟菱形藻的形态特征、生长特性与毒素检测	20
3.2.1 形态结构.....	20
3.2.2 培养条件对尖刺拟菱形藻生长的影响.....	26

3.2.3 尖刺拟菱形藻软骨藻酸检测与分析.....	35
3.3 尖细拟菱形藻的形态特征、生长特性与毒素检测	39
3.3.1 形态结构.....	39
3.3.2 培养条件对尖细拟菱形藻生长的影响	42
3.3.3 尖细拟菱形藻软骨藻酸检测与分析.....	48
3.4 多纹拟菱形藻的形态特征、生长特性及毒素检测	50
3.4.1 形态结构.....	50
3.4.2 培养条件对多纹拟菱形藻生长的影响	52
3.4.3 多纹拟菱形藻软骨藻酸检测与分析.....	56
3.5 巴西拟菱形藻的形态特征、生长特性及毒素检测	57
3.5.1 形态结构.....	57
3.5.2 培养条件巴西拟菱形藻生长的影响	58
3.5.3 巴西拟菱形藻软骨藻酸检测与分析.....	62
3.6 加拿大多列拟菱形藻的形态特征、生长特性及毒素检测	62
3.6.1 形态结构.....	62
3.6.2 常规培养对多列拟菱形藻生长的影响.....	69
3.6.3 多列拟菱形藻软骨藻酸检测与分析.....	72
3.7 常规培养五种拟菱形藻生长差异性比较分析	79
3.8 几种分离自中国东南沿海的羽纹纲硅藻的产毒可能性分析	80
第四章 讨论	82
4.1 拟菱形藻形态与产毒的关系	82
4.2 拟菱形藻生长与产毒的关系	83
4.3 不同培养条件对拟菱形藻产毒的影响	83
4.4 本研究未检测到软骨藻酸的可能原因	86
第五章 总结与展望	88
5.1 进一步开展对中国海域拟菱形藻是否产毒的确认工作	88
5.2 对潜在产毒拟菱形藻不产毒机理的探究	89
5.3 拟菱形藻种质保存	89
参考文献.....	90

攻读硕士阶段发表的论文和参加的课题	99
参加的课题	99
致谢	100

厦门大学博硕士论文摘要库

Contents

Chinese Abstract	I
English Abstract	II
Chapter1 Preface	1
1 The historical and current studies on <i>Pseudo-nitzschia</i>	1
2 The source, characters and the testing method of neurotoxin domoic acid(DA)	1
2.1 The source of DA	1
2.2 The characters of DA	2
2.3 The testing methods of DA	4
3 The current study on <i>Pseudo-nitzschia</i> and domoic acid	5
3.1 DA pollution induced by toxic <i>Pseudo-nitzschia</i>	5
3.2 Factors affecting <i>Pseudo-nitzschia</i> to produce DA	7
4 The current study on the <i>Pseudo-nitzschia</i> in China	10
5 The content, goal and the significance of the study	12
Chapter 2 Materials and Methods	13
2.1 Materials	13
2.2 Methods	14
2.2.1 Isolation of <i>Pseudo-nitzschia</i>	14
2.2.2 Culture of <i>Pseudo-nitzschia</i>	14
2.2.3 Samples treatment and analysis	15
2.3 Data analysis	18
2.4 Instruments and equipments	19
Chapter 3 Results and Analysis	20
3.1 The isolation and purification of <i>Pseudo-nitzschia</i>	20
3.2 The morphological growth study and DA test of <i>P. pungens</i>	20
3.2.1 The morphological study	20
3.2.2 The results of growth study of <i>P. pungens</i>	26
3.2.3 DA test of <i>P. pungens</i>	35

3.3 The morphological, growth study and DA test of <i>P. cupidata</i>	39
3.3.1 The morphological study	39
3.3.2 The results of growth study of <i>P. cupidata</i>	42
3.3.3 DA test of <i>P. cupidata</i>	48
3.4 The morphological, growth study and DA test of <i>P. multistriata</i>	50
3.4.1 The morphological study	50
3.4.2 The results of growth study of <i>P. multistriata</i>	52
3.4.3 DA test of <i>P. multistriata</i>	56
3.5 The morphological, growth study and DA test of <i>P. brasiliana</i>	57
3.5.1 The morphological study	57
3.5.2 The results of growth study of <i>P. brasiliana</i>	58
3.5.3 DA test of <i>P. brasiliana</i>	62
3.6 The morphological, growth study and DA test of <i>P. multiseries</i>	62
3.6.1 The morphological study	62
3.6.2 The growth study of <i>P. multiseries</i>	69
3.6.3 The DA test by HPLC	72
3.7 Analysis of five <i>Pseudo-nitzschia</i> genus cultivated in normal conditions	79
3.8 Analysis of possible toxin producer of pennate diatoms isolated from Southeast China Sea	80
Chapter 4 Discussion	82
4.1 The relationship between morphology and DA production	82
4.2 The relationship between growth and DA production	83
4.3 The effect of different cultivated conditions on DA production	83
4.4 The possible reasons of no DA were test in the study	86
Chapter 5 Conclusion and Prospect	88
References	90
Acknowledgements	100

厦门大学博硕士论文摘要库

中文摘要

本论文对分离自中国东南沿海的 4 种 (15 株) 拟菱形藻进行了形态学, 不同培养条件下的生长与毒素检测的研究, 初步探讨了中国海域拟菱形藻未发现可检出毒素的原因; 对可产毒种类—多列拟菱形藻 (*Pseudo-nitzschia multiseries*, 加拿大株系) 进行了形态学、生长和毒素检测与比较, 分析了不同株系多列拟菱形藻毒素产量的差异和不同生长周期对毒素产生的影响; 对分离自中国东南沿海的 13 种 (21 株) 其它羽纹纲硅藻进行了软骨藻酸 (Domoic Acid, DA) 检测研究, 分析了中国海域贝类体内发现毒素的可能原因。取得的主要结果如下:

利用微吸管法从中国东南沿海不同水域分离得到 4 种 (15 株) 拟菱形藻, 利用光学显微镜和透射电镜进行了形态学研究, 鉴定为尖刺拟菱形藻 (*P. pungens*)、尖细拟菱形藻 (*P. cuspidata*)、多纹拟菱形藻 (*P. multistriata*) 和巴西拟菱形藻 (*P. brasiliiana*), 其中除巴西拟菱形藻外, 其它三种在国外均有产毒报道。

以细胞密度、叶绿素 a 含量及生长速率三种因子为指标, 对 5 种拟菱形藻进行了不同条件培养下生长的研究与比较, 结果显示, 在常规培养条件下, 不同株系间在生长上表现出显著差异, 验证了来源地不同的同种拟菱形藻间地理差异的存在; 对同一株系, 不同条件下的培养也显示出显著差异的存在, 说明外界环境因子的改变, 会影响拟菱形藻的生长特性。

通过设置不同的光周期和硅、磷营养盐限制条件, 对从中国东南沿海分离得到的 4 种拟菱形藻进行了常规培养和诱导培养实验, 采用高效液相色谱法 (HPLC) 对各个生长周期的培养藻液进行了检测, 均未发现可检出 DA 存在, 说明本实验所用的 4 种拟菱形藻在上述培养条件下, 均不能产生可检出 DA。

对 Bates Stephen (加拿大 Bedford 海洋研究所) 提供的加拿大有毒拟菱形藻 *P. multiseries* 进行了常规条件培养, 比较研究了与中国不同藻种的生长差异, 进行了毒素检测和产量分析, 验证了 DA-HPLC 方法的可行性, 并为下一步对中国水域拟菱形藻与国外产毒拟菱形藻之间进行对比, 寻找其不产毒的原因提供了前期工作基础。

对实验室现有的 13 种 (21 株) 分离自中国东南沿海的羽纹纲硅藻进行了 DA-HPLC 检测, 均未发现可检出 DA 存在, 说明本实验室藻种库现有的 21 株羽纹纲硅藻可能不能产生 DA。

关键词: 拟菱形藻; 生长; 软骨藻酸检测.

Abstract

This dissertation mainly focuses on the studies on four species of genus *Pseudo-nitzschia* isolated from Southeast China Sea and the studies include the morphological study, growth study under different conditions, toxin (Domoic Acid) determination and the primary exploration of the reasons why the *Pseudo-nitzschia* distributed in China Sea cannot produce DA. The morphological study, growth and toxin (Domoic Acid) determination on the six strains of *P. multiseries* (from Canada) were also carried out. The different DA yields between the *P. multiseries* strains and different cell cycle are analyzed. In addition, the toxin (Domoic Acid) determination were also carried out on thirteen pennate species (twenty-one strains) isolated from China coastal sea area and the possible reasons of the toxin in seashells were discussed. The results are as followed:

Thirteen strains of *Pseudo-nitzschia* isolated from different locations in Southeast China Sea by microtube method. These thirteen strains *Pseudo-nitzschia*, are identified as four species that are *P. pungens*, *P. multiserita*, *P. cuspidata* and *P. brasiliensis* by light microscope and electron microscope. Except the *P. brasiliensis*, the rest have been reported to be toxin producers.

Taking the cell density, Chl *a* and growth rate as the indicators, four species of *Pseudo-nitzschia* that are isolated from different locations of Southeast China Sea were studied by being cultivated respectively under normal conditions and different inducing conditions. The results suggested that the strains isolated from different locations have differences in growing conditions under the normal cultivating conditions, which supported that the same species from different geographical areas do have differences. And as for the same species, the differences do exist under the different cultivating conditions, which showed that the changes of environmental factors can influence the growth characteristics.

Deficiency of silica and phosphorus, different photoperiods were set as the inducing cultivation. According to the HPLC measurement, no DA was determined under the set cultivating conditions, which lead to the conclusion that these four

species of *Pseudo-nitzschia* under the study cannot produce DA either under the normal conditions or inducing conditions.

Under the normal conditions, the growth study between the strains, the toxin (Domoic Acid) determination and DA yield analysis were also carried out on the *P. multiseries* provided by the Bates Steven, (Bedford institution of oceanography, Canada). The results approve the testing method applied in this study is feasible and accurate. And what is more, the result provide the rudimentary data for further study on the comparison between the DA producers and no DA producers, and the laid the foundation for exploration of the reasons why the *Pseudo-nitzschia* distributed in China Sea area cannot produce DA.

DA-HPLC was also carried out on 21 strains which belong to 13 species of pennate diatoms isolated from China Sea. The results showed that 21 species of pennate diatoms cannot produce tested DA.

Key words: *Pseudo-nitzschia*; growth; domoic acid analysis.

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